Support in the specification for the amendment of the claims:

Claim 1, step b, is amended to recite removing the nuclear DNA from a recipient oocyte, transferring the nucleus from said donor cell into the recipient oocyte, and generating an embryo. Support for the added element of removing the nuclear DNA from the recipient oocyte is found in the specification, for example, at page 4, lines 9-12, in which enucleation of a recipient oocyte is described.

Claim 1, step e, is amended to recite examining the injection sites for teratoma formation and signs of rejection of the injected cells, or of teratomas derived therefrom. Support for the added element of examining the injection sites signs of rejection of the injected cells, or of teratomas derived therefrom, is found in the specification, for example, at page 5, lines 23-24, which describes a method comprising injecting stem cells capable of forming teratomas into an animal and examining the injection sites for signs teratoma formation and signs of subsequent rejection.

Claim 38 is amended to recite a non-human, non-immune-compromised animal containing at least one teratoma produced from a cloned cell. Support for the added elements of the claimed animal being a non-human, non-immune-compromised animal is found in the specification, for example, in the paragraph bridging pages 5-6, which describes using a donor animal capable of mounting an immune response that causes rejection of transplanted cells that are not immune compatible, and in the paragraph at the bottom of page 6, which describes using a non-human animal in the disclosed assay.

Claim 48 is amended to depend on claim 44; its dependence on itself was clearly typographical error.

Rejection of the claims under 35 U.S.C. § 101 for reciting non-statutory subject matter:

Claims 38 and 41-43 of the application are rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter, because they encompass a human being. Claim 38 is amended to recite a non-human animal; accordingly, withdrawal of the rejection is respectfully requested.

Rejection of the claims under 35 U.S.C. § 101 for provisional double patenting:

Claims 1-14, 20-28, 33, and 38-48 were provisionally rejected under 35 U.S.C. § 101 as being directed to the same invention as claims of U.S. Application No. 09/797,684. As

stated above, claims 8-10, 20-28, and 33 are cancelled. Therefore, claims 1-7, 11-14, and 38-48 remain subject to the rejection for provisional double patenting. The amendment of independent claims 1 and 38 directs the remaining claims to subject matter that is not identical to that recited in the claims of U.S. Application No. 09/797,684. The Applicants therefore respectfully request withdrawal of the provisional double patenting rejection.

Rejection of the claims under 35 U.S.C. § 112, first paragraph:

Claims 1-14, 20-28, and 33 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that is not enabled by the specification. As stated above, claims 8-10, 20-28, and 33 are cancelled. Therefore, claims 1-7 and 11-14 remain subject to the rejection under 35 U.S.C. §112, first paragraph. The rejection states the only type of recipient cell that is enabled by the specification is an oocyte. It further states that it is well-known that nuclear transfer cloning includes the steps of enucleating the recipient cell, fusing the donor cell to the recipient cell, and activating the resulting nuclear transfer unit to generate an embryo, and that claim 1 must recite these steps as well.

Claim 1 is amended to recite enucleating an oocyte and using it as the recipient cell; however, the Applicants respectfully traverse the requirement that the recited step of cloning by nuclear transfer be expanded to recite fusing the donor cell to the recipient cell, and activating the resulting nuclear transfer unit to generate the embryo. Nuclear transfer cloning has become a commonly used technique in the art of animal cloning. While nuclear transfer cloning is routinely performed by a method that comprises fusing the donor cell to the recipient cell, those in the art recognize that it can also be performed successfully by removing the nucleus of the donor cell and injecting the donor nucleus into the recipient cell, a technique that does not require the step of fusing the donor and recipient cell membranes. Similarly, those skilled in the art recognize that the step of activation of a nuclear transfer unit to generate an embryo can be carried out by a variety of means, including the passive step of incubating the nuclear transfer unit so that it activates spontaneously. The invention recited in claim 1 is a method for testing the immune compatibility of cloned cells or tissues in an animal model - an invention that includes but goes well beyond the production of a nuclear transfer embryo. The Applicants respectfully submit that it would be improper to require that the pending claims be limited to a method that uses one particular method for producing a nuclear transfer embryo out of the various combinations of methods for making nuclear transfer embryos that are known in the art. The Applicants submit that the

specification fully enables one skilled in the art to use the invention as claimed, and respectfully request reconsideration and withdrawal of the enablement rejection under 35 U.S.C. §112, first paragraph.

Rejection of the claims under 35 U.S.C. § 112, second paragraph:

Claims 1-14, 21, 22, 29, 30, 33, and 48 were rejected under 35 U.S.C. § 112, second paragraph. As stated above, claims 8-10, 20-28, and 33 are cancelled. Therefore, claims 1-7, 11-14, and 48 remain subject to the rejection under 35 U.S.C. §112, second paragraph.

Claim 1 was rejected under 35 U.S.C. § 112, second paragraph, on the ground that it is incomplete, because the final step of the recited assay - examining for teratoma formation - does not relate to testing for immune compatibility. The specification teaches that transplanted embryonic cells and the teratomas they form are expected to elicit an immune response and be rejected if they are not histocompatible with the transplant recipient. The phenomenon of rejection of a non-histocompatible cell or tissue graft is well-known to those skilled in the art. A person who is skilled in the art would understand that the step of examining for teratoma formation recited in claim 1 is a test for immune compatibility, because he/she would reasonably expect the formation of teratomas by the injected cells to be inhibited by the rejection response elicited by injection of non-histocompatible cells. Nonetheless, step e of claim 1 is amended to recite examining the injection sites for teratoma formation and signs of rejection of the injected cells or of teratomas derived therefrom. The added element clearly relates the last step of the claimed method to testing for immune compatibility; accordingly, the Applicants respectfully request withdrawal of the rejection of claim 1 under 35 U.S.C. §112, second paragraph, as being incomplete.

Claim 1 was also rejected under 35 U.S.C. § 112, second paragraph, on the ground that recitation of "other recipient cell" was indefinite. As amended above, claim 1 does not recite an "other recipient cell;" accordingly, the Applicants respectfully request withdrawal of the rejection of claim 1 under 35 U.S.C. §112, second paragraph, on this ground as well.

Claim 48 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for depending on itself. Claim 48 is amended to depend on claim 44; and, the Applicants respectfully request withdrawal of the rejection of claim 48 under 35 U.S.C. §112, second paragraph.

Rejection of the claims under 35 U.S.C. § 102(b):

Claims 38, 42, and 44-47 were rejected under 35 U.S.C. § 102(b) as being anticipated by Anderson et al., which discloses transplanting pluripotent bovine and porcine embryonic cells into immune-compromised (athymic) mice to form teratomas. Claim 38 is amended to recite a non-human, non-immune-compromised animal containing at least one teratoma produced from a cloned cell. Anderson et al. do not disclose transplanting cloned pluripotent embryonic cells into a non-immune-compromised animals to form a teratoma. The Applicants therefore respectfully request withdrawal of the rejection of claims 38, 42, and 44-47 under 35 U.S.C. §102 (b) as being anticipated by Anderson et al.

All issues raised by the Office Action dated March 10, 2002, have been addressed in this Reply. It is respectfully submitted that the present application is in a condition for allowance and a Notice to that effect is earnestly solicited. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that he contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,

PILLSBURY WINTHROP LLP

Date: September 10, 2002

By: _____

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Enclosures:
- substitute specification
- copy of abstract of Ruffing et al., Biol. Reprod. (1993) 48(4):889-904

APPENDIX

The text and claims are amended as shown below:

IN THE SPECIFICATION:

The paragraph beginning on line 30 of page 4 (line 1 of page 5 of the substitute specification) is amended as follows:

-- Thus, mitochondrial peptides displayed at the cell surface can serve as histocompatibility antigens, seeing as two separate systems have been identified in mice and rats, respectively. There is no reason to believe that similar systems would not be present in other mammals. Therefore, foreign mitochondria would be expected to result in the rejection of therapeutic tissue generated by nuclear transfer technology. Instead, using the methods of the present invention, the present inventors have surprisingly found in performing the methods of the present invention that nuclear transfer generated cells having allogeneic mitochondria are not rejected when transplanted into the nuclear donor. --

The paragraph beginning on line 10 of page 7 (line 17 of page 7 of the substitute specification) is amended as follows:

-- For instance, according to a report in the New York Times on November 12, 1998 (Nicholas Wade, "Human Cells Revert to Embryo State, Scientists Assert"), although cow mitochondria would not be expected to work with a human nucleus, the mitochondria of chimpanzees and gorillas would be expected to be functional in human cells. In fact, [as noted on the website www.globalchange.com,] scientists have already made chimeric "geep" (combined sheep and goat), and "camas" (combined camels and lamas), suggesting that the cells and cellular organelles of closely related species would be functionally compatible (see Ruffing et al., Biol. Reprod. (1993) 48(4):889-904; also "Bush telegraph on chimeras," The Daily Telegraph, January 22, 1998, p. 27; "It's a geep: cross-breeding goats and sheep," Time, February 27, 1984, p. 71; "Meet the geep: part goat – part sheep," Science, May 1984, 5: 6). According to Jakovcic et al. (1975, "Sequence homology between mitochondrial DNAs of different eukaryotes," Biochem. 14(10): 2043- 50), evolutionary divergence of mtDNA sequences appears to have occurred at rates similar to that for unique sequence nuclear DNA. --

The paragraph beginning on line 10 of page 9 (line 21 of page 9 of the substitute specification) is amended as follows:

-- The ability to re-clone cloned mammals and generate a line of cloned mammals that are isogenic for both nuclear and mitochondrial DNA allows for concurrent injection of the cross-species cloned cells containing allogeneic mitochondria into separate mammals, thereby facilitating the retrieval of panels of antibodies and lymphocytes specific for different mitochondrial backgrounds. Methods of recloning cloned mammals based on the observation that nuclear transfer can be used to rejuvenate senescent cells are disclosed in commonly assigned, copending Application Serial No. [_____] 09/656,173, filed concurrently herewith and incorporated by reference in its entirety. Of course, it is also possible to generate cloned mammals having isogenic mitochondrial DNA by performing nuclear transfer from a single donor using multiple oocytes or other suitable recipient cells from a single recipient mammal or cell line. Thus the methods of the present invention may also be performed wherein said discs and/or stem cells are injected into separate mammals which are isogenic to the nuclear donor with respect to both nuclear and mitochondrial DNA in order to isolate panels of antibodies and/or lymphocytes. --

The paragraph beginning on line 6 of page 12 (line 21 of page 12 of the substitute specification) is amended as follows:

-- In particular, the immune-compatible tissues and cells generated are useful in methods of providing a patient in need of a transplant with an immune-compatible transplant. Such a method further comprises, in addition to the above steps, transplanting said engineered tissue into a patient. The fact that the present inventors have surprisingly found that cloned cells containing isogenic nuclear DNA and allogeneic mitochondrial DNA do not induce transplant rejection has particular relevance for transplants which replace native cells suffering from mitochondrial damage, for instance as in [amythrophic] amyotrophic lateral sclerosis (ALS), or Leber's hereditary optic neuropathy (LHON). In such cases, cloned tissue having isogenic nuclear DNA and allogeneic mitochondrial DNA that does not induce an immune reaction is the most ideal tissue for transplantation in that such tissue not only

provides the closest histocompatibility match, but it also effectuates mitochondrial gene therapy in that tissue containing damaged mitochondria is replaced. --

The paragraph beginning on line 5 of page 15 (line 26 of page 15 of the substitute specification) is amended as follows:

-- In this regard, it is pertinent to note that the present inventors have also discovered that the cloning procedures of the present invention enables the rejuvenation of senescent cells, thereby foregoing any concerns regarding the genetic age of cloned tissues. The disclosure of U.S. application Serial No. [09/_____] 09/656,173, which is co-owned with the present application, reports the inventors' surprising observations relating to the rejuvenation of primary cells using nuclear transfer, and is herein incorporated in its entirety. The finding that the cloning process rejuvenates older cells is particularly relevant for designing therapeutic tissues expressing more than one heterologous gene, or having more than one gene knocked out, because such tissues can be generated by cloning and re-cloning primary cells of the same genetic background. --

The paragraph beginning on line 2 of page 23 (line 5 of page 24 of the substitute specification) is amended as follows:

-- Five micron sections of formalin fixed paraffin embedded tissue were cut and stained with hematoxylin and eosin (H&E). Immunocytochemical analyses were performed using specific antibodies in order to identify the cell type of the retrieved tissues. Histochemical analyses using aldehyde fuschin-alcian blue, and immunocytochemical studies using monoclonal anti-collagen II (Chemicon, St. Louis, MO) were used to identify the engineered cartilage structures. Monoclonal sarcomeric tropomyosin (Sigma, St. Louis, MO) and troponin I (Chemicon, Temecula, CA) antibodies were used to detect skeletal and cardiac muscle fibers, respectively. Immunolabeling was performed using the avidin-biotin detection system. Sections were counterstained with methyl green. --

IN THE CLAIMS:

Please cancel claims 8-10, 20-28, and 33, and amend claims 1, 38, and 48 as shown below:

1. A method of testing the immune compatibility of cloned cells or tissues in an animal model, comprising:

a. obtaining a cell from a donor animal;

b. removing the nuclear DNA from a recipient oocyte, transferring the nucleus from said donor cell into [a] the recipient oocyte [or other suitable recipient cell to generate], and generating an embryo;

c. isolating an embryo having at least one cell, an embryonic disc and/or stem cell from said embryo;

d. injecting said embryo, disc and/or stem cell into said donor animal at the same time as control embryonic disc and/or stem cell; and

e. examining the injection sites for teratoma formation and signs of rejection of the injected cells, or of teratomas derived therefrom.

38. (Amended) [An] A non-human, non-immune-compromised animal containing at least one teratoma produced from a cloned cell.

48. The teratoma of Claim [48] 44, wherein said teratoma comprises cloned cells having isogenic nuclear DNA and allogeneic mitochondrial DNA, or a mixture of allogeneic and isogenic mitochondrial DNA.

1: Biol Reprod 1993 Apr;48(4):889-904

Effects of chimerism in sheep-goat concepti that developed fro blastomere-aggregation embryos.

Ruffing NA, Anderson GB, Bondurant RH, Currie WB, Pashen RL.

Department of Animal Science, University of California, Davis 95616-8521.

Chimeric sheep-goat pregnancies were established in 24 ewes and 29 does by transferring 251 embryos, prepared by the blastomere-aggregation technique, to 52 ewes and 61 does. Fifteen does experienced early pregnancy failure; however, term offspring were delivered by 24 ewes (17 lambs, 3 kids, 6 chimeras) and 14 does (6 lambs, 9 kids, 6 chimeras). (Fetal classifications were based on phenotype, red blood cell isozymes, and lymphocyte antigen expression). RIAs for ovine and caprine placental lactogen detected chimerism in the binucleate cell population of the trophoblast throughout the pregnancies of 2 ewes and 7 does; these pregnancies resulted in the birth of 12 healthy offspring. Histological examinations of intact placentomes from 2 of these recipients revealed a continuous cellular trophoblast apposed to a syncytium as in normal placentas. Chimerism was detected electrophoretically in the membranes of the placentas with binucleate cell chimerism and in 17/28 of the other placentas. Data collected on placental lactogen production, chimerism in the conceptus, and placental morphometry were examined with respect to the stages of the blastomeres aggregated to form the chimeric embryo and with respect to fetal status at delivery. For comparison, analogous data were collected on sheep-goat concepti that developed from embryos prepared by inner cell mass transplantation.

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